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(54) Title: HUMAN RETINOID X RECEPTOR - GAMMA (hRXR-GAMMA)

(57) Abstract

The present invention relates to a novel retinoid receptor, human retinoid X receptor γ. hRXRγ modulates transcription of certain genes in the presence of certain retinoid compounds. hRXRγ differs from known retinoid receptors in nucleotide sequence, amino acid sequence, and expression pattern in tissues. The invention provides isolated, purified, or enriched nucleic acid encoding hRXRγ polypeptides and vectors containing thereof, cells transformed with such vectors, and methods of screening for compounds capable of binding hRXRγ polypeptides. The invention also provides isolated, purified, enriched, or recombinant hRXRγ polypeptides, antibodies having specific binding affinity to hRXRγ polypeptides, and hybridomas producing such antibodies.

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HUMAN RETINOID X RECEPTOR - GAMMA (hRXR-GAMMA)

Field of the Invention

This invention relates to the cloning and uses of a human retinoid X receptor subtype.

Background of the Invention

Retinoic acid is a vitamin A metabolite which has been recognized as inducing a broad spectrum of biological effects. A variety of structural analogues of retinoic acid have been synthesized that also have been found to be bioactive. Some, such as Retin-A® (registered trademark of Johnson & Johnson) and Accutane® (registered trademark of Hoffmann-LaRoche), have found utility as therapeutic agents for the treatment of various pathological conditions. Metabolites of vitamin A and their synthetic analogues are collectively herein called "retinoids". Synthetic retinoids have been found to mimic many of the pharmacological actions of retinoic acid. However, the broad spectrum of pharmacological actions of retinoic acid is not reproduced in full by all bioactive synthetic retinoids.

Medical professionals are interested in the medicinal applications of retinoids. Among their uses approved by the FDA is the treatment of severe forms of acne and psoriasis. Evidence also exists that these compounds can be used to arrest and, to an extent, reverse the effects of skin damage arising from prolonged exposure to the sun. Other evidence indicates that these compounds may be useful in the treatments of a variety of cancers including melanoma, cervical cancer, some forms of leukemia, and basal and squamous cell carcinomas. Retinoids have also been shown to be efficacious in treating premalignant cell lesions, such as oral leukoplakia, and to prevent the occurrence of malignancy.

Retinoids are able to cross passively biological membranes and control cell functions by using specific

intracellular receptors as signal transducers. These intracellular receptors, located in the nucleus in the presence of their retinoid ligands, function as ligand-activated transcription factors that modulate gene expression through binding to specific DNA sequences located in the regulatory regions of target genes.

Retinoids regulate the activity of two distinct intracellular receptor subfamilies; the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). The RAR and RXR subfamilies are divided into six subtypes, based upon their primary sequence homology, their ability to bind to various retinoid analogues, and by their promoter recognition sequence specificity (Mangelsdorf DJ, Umesono K, and Evans RM 1994 Retinoid receptors. In: Sporn MB, Roberts AB, and Goodman DS (eds) The Retinoids: Biology, Chemistry, and Medicine. Raven Press, pp 319-349; Giguere V, Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signalling. Endocrine Reviews 15:61-79, 1994). The RARs have three subtypes denoted α, β, and γ. The RXRs also have three known subtypes, α, β, and γ.

On one hand, RARs and RXRs share common structure and functional domains with other members of the steroid hormone receptor superfamily, comprising an amino-terminal region of variable length, a DNA-binding domain located in the central region, and a ligand-binding domain encompassing most of the carboxy-terminal end of the proteins. On the other hand, RARs and RXRs differ in several aspects. First, the RARs and RXRs are divergent in primary structure, e.g., the ligand-binding domains of RARα and RXRα have only approximately 27% amino acid identity (i.e., "homology"). These structural differences are reflected in the different relative degrees of responsiveness of RARs and RXRs to various vitamin A metabolites and synthetic retinoids.

RARs bind to both 9-cis retinoic acid (9cRA) and all-trans retinoic acid (tRA) with equally high affinity,

displaying K_d values of 0.2 - 0.8 nM (Allenby G, et al., 1993, "Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids." Proc Natl Acad Sci USA 90:30-34; Allegretto EA, et al., 1993, "Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: correlation with hormone binding and effects of metabolism." J. Biol. Chem. 268:26625-26633). RXRs bind with high affinity and specificity to 9cRA (Levin AA, et al., 1992, 9-cis retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. Nature 355:359-361; Heyman RA, et al., 1992, 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. Cell 68:397-406) with K_d values of 1-2 nM (Allegretto EA, et al., 1993, "Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: correlation with hormone binding and effects of metabolism." J. Biol. Chem. 268:26625-26633), but do not bind to tRA (IC_{50} > 50,000 nM versus tritiated 9cRA (Allenby G, et al., 1993, "Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids." Proc. Natl. Acad. Sci. USA. 90:30-34; Allegretto EA, et al., 1993, "Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: correlation with hormone binding and effects of metabolism." J. Biol. Chem. 268:26625-26633).

In addition, distinctly different patterns of tissue distribution are seen for RARs and RXRs. For example, in contrast to the RARs, which are not expressed at high levels in the visceral tissues, RXRα mRNA has been shown to be most abundant in the liver, kidney, lung, muscle and intestine.

Furthermore, RARs and RXRs have different target gene specificity. For example, response elements in cellular retinol binding protein type II (CRBPII) and apolipoprotein AI genes confer responsiveness to RXR, but not to RAR. RAR has also been shown to repress RXR-mediated

activation through the CRBPII RXR response element (Mangelsdorf et al., Cell, 66:555-61 (1991)).

The RXR class of retinoid receptors not only function as effector molecules for 9-*cis* RA but also function as heterodimeric partners for other members of the intracellular receptor superfamily including RARs, the thyroid hormone receptor, the peroxisome proliferator-activator receptor (PPAR), the vitamin D receptor, and a number of other intracellular receptors whose ligands have not yet been identified (orphan receptors) (Leid,M., Kastner,P., Lyons,R., Nakshatri,H., Saunders,M., Zacharewski,T., Chen,J.Y., Staub,A., Garnier,J.M., Mader,S. and Chambon,P. (1992) Cell, **68**, 377-395, Yu,V.C., Delsert,C., Andersen,B., Holloway,J.M., Devary,O.V., Naar,A.M., Kim,S.Y., Boutin,J.M., Glass,C.K. and Rosenfeld,M.G. (1991) Cell, **67**, 1251-1266, Kliewer,S.A., Umesono,K., Mangelsdorf,D.J. and Evans,R.M. (1992) Nature, **355**, 446-449, Kliewer,S.A., Umesono,K., Noonan,D.J., Heyman,R.A. and Evans,R.M. (1992) Nature, **358**, 771-774, Kliewer,S.A., Umesono,K., Heyman,R.A., Mangelsdorf,D.J., Dyck,J.A. and Evans,R.M. (1992) Proc. Natl. Acad. Sci. U. S. A., **89**, 1448-1452). In fact, RXR-β was first identified in either human or rat cells biochemically by a number of laboratories using functional assays to characterize protein molecules that increased the DNA binding properties of VDR, RAR, TR, and H2BPII (Hamada,K., Gleason,S.L., Levi,B.Z., Hirschfeld,S., Appella,E. and Ozato,K. (1989) Proc. Natl. Acad. Sci. U. S. A., **86**, 8289-8293). Upon isolation and cloning of these molecules it became evident that these molecules were the human counterparts of mouse RXR-β.

Some members of the RXR family of receptors have been described in humans, rat, chicken (Rowe,A., Eager,N.S. and Brickell,P.M. (1991) Development, **111**, 771-778) and xenopus (Blumberg,B., Mangelsdorf,D.J., Dyck,J.A., Bittner,D.A., Evans,R.M. and De Robertis,E.M. (1992) Proc. Natl. Acad. Sci. U. S. A., **89**, 2321-2325). To date

only two subtypes of RXR receptors, α and β, have been characterized from humans (Mangelsdorf,D.J., Ong,E.S., Dyck,J.A. and Evans,R.M. (1990) Nature, **345**, 224-229, Fleischhauer,K., Park,J.H., DiSanto,J.P., Marks,M., Ozato,K. and Yang,S.Y. (1992) Nucleic. Acids. Res., **20**, 1801, Leid,M., Kastner,P., Lyons,R., Nakshatri,H., Saunders,M., Zacharewski,T., Chen,J.Y., Staub,A., Garnier,J.M., Mader,S. and Chambon,P. (1992) Cell, **68**, 377-395).

Summary of the Invention

The lack of a human RXR-γ cDNA clone has hampered research such as an examination of the expression patterns of the RXR family of receptors in human tissues and cell lines. To alleviate this problem applicant cloned and characterized a human RXR-γ subtype cDNA.

The present invention relates to hRXR-γ polypeptides, nucleic acids encoding such polypeptides, cells, tissues and animals containing such polypeptides and nucleic acids, antibodies to such polypeptides, assays utilizing such polypeptides and nucleic acids, and methods relating to all of the foregoing. The hRXR-γ polypeptides, nucleic acids, and antibodies are useful for establishing the tissue specific expression pattern of hRXR-γ gene. For example, a Northern blot can be used to reveal tissue specific expression of the gene. They are also useful for screening compounds (e.g., compounds active as primary endogenous inducers of the hRXR-γ polypeptides) for improved pharmacological profiles for the treatment of diseases with higher potency, efficacy, and fewer side effects.

The present invention is based upon the identification and isolation of a novel human retinoid X receptor subtype termed hRXR-γ that is activated by binding of 9-cis retinoic acid or LG100069, i.e., (*E*)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]

benzoic acid. hRXR-γ has 463 amino acids and a predicted molecular weight of 55 kD.

Thus, in a first aspect the invention features an isolated, purified, enriched or recombinant nucleic acid encoding a hRXR-γ polypeptide.

By "isolated" in reference to nucleic acid is meant a polymer of 2 (preferably 21, more preferably 39, most preferably 75) or more nucleotides conjugated to each other, including DNA or RNA that is isolated from a natural source or that is synthesized. The isolated nucleic acid of the present invention is unique in the sense that it is not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only nucleotide chain present, but does indicate that it is the predominate sequence present (at least 10 - 20% more than any other nucleotide sequence) and is essentially free (about 90 - 95% pure at least) of non-nucleotide material naturally associated with it. Therefore, the term does not encompass an isolated chromosome encoding a hRXR-γ polypeptide.

By "enriched" in reference to nucleic acid is meant that the specific DNA or RNA sequence constitutes a significantly higher fraction (2 - 5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reduction in the amount of other DNA or RNA present, or by a preferential increase in the amount of the specific DNA or RNA sequence, or by a combination of the two. However, it should be noted that enriched does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased in

a useful manner and preferably separate from a sequence library. The term "significantly" here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other nucleic acids of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there is no DNA or RNA from other sources. The DNA from other sources may, for example, comprise DNA from a yeast or bacterial genome, or a cloning vector such as pUC19. This term distinguishes from naturally occurring events, such as viral infection, or tumor type growths, in which the level of one mRNA may be naturally increased relative to other species of mRNA. That is, the term is meant to cover only those situations in which a person has intervened to elevate the proportion of the desired nucleic acid.

By "purified" in reference to nucleic acid does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, *e.g.*, in terms of mg/ml). Individual clones isolated from a cDNA library may be purified to electrophoretic homogeneity. The claimed DNA molecules obtained from these clones could be obtained directly from total DNA or from total RNA. The cDNA clones are not naturally occurring, but rather are preferably obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The construction of a cDNA library from mRNA involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection of the cells carrying the cDNA library. Thus, the process which includes the construction of a cDNA library from mRNA and isolation of distinct cDNA clones yields an approximately 10^6-fold purification of the native message. Thus, purification of at least one

order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

By "a hRXR-γ polypeptide" is meant two or more contiguous amino acids set forth in the full length amino acid sequence of SEQ ID NO:2, wherein said contiguous amino acids have a sequence different from those of mouse RXR-γ polypeptides. The hRXR-γ polypeptide can be encoded by a full-length nucleic acid sequence or any portion of the full-length nucleic acid sequence, so long as a functional activity of the polypeptide is retained.

In preferred embodiments the isolated nucleic acid comprises, consists essentially of, or consists of a nucleic acid sequence set forth in the full length nucleic acid sequence SEQ ID NO:1 or at least 27, 30, 35, 40 or 50 contiguous nucleotides thereof and the hRXR-γ polypeptide comprises, consists essentially of, or consists of at least 9, 10, 15, 20, or 30 contiguous amino acids of a hRXR-γ polypeptide.

By "comprising" is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

Compositions and probes of the present invention may contain human nucleic acid encoding a hRXR-γ polypeptide but are substantially free of nucleic acid not encoding a human hRXR-γ polypeptide. The human nucleic acid encoding a hRXR-γ polypeptide is at least 18 contiguous bases of the nucleotide sequence set forth in SEQ. ID NO. 1 and will selectively hybridize to human genomic DNA encoding a hRXR-γ polypeptide, or is complementary to such a sequence. The nucleic acid may be isolated from a natural source by cDNA cloning or subtractive hybridization; the natural source may be blood, semen, and tissue of humans; and the nucleic acid may be synthesized by the triester method or by using an automated DNA synthesizer. In yet other preferred embodiments the nucleic acid is a unique region, for example those useful for the design of hybridization probes to facilitate identification and cloning of additional polypeptides, the design of PCR probes to facilitate cloning of additional polypeptides, and obtaining antibodies to polypeptide regions.

By "unique nucleic acid region" is meant a sequence present in a full length nucleic acid coding for a hRXR-γ polypeptide that is not present in a sequence coding for any other naturally occurring polypeptide. Such regions preferably comprise 12 or 20 contiguous nucleotides present in the full length nucleic acid encoding a hRXR-γ polypeptide.

The invention also features a nucleic acid probe for the detection of a hRXR-γ polypeptide or nucleic acid encoding a hRXR-γ polypeptide in a sample. The nucleic acid probe contains nucleic acid that will hybridize to a sequence set forth in SEQ ID NO:1, but not to a mouse RXR-γ nucleic acid sequence under high stringency hybridization conditions. In preferred embodiments the nucleic acid probe hybridizes to nucleic acid encoding at least 12, 27, 30, 35, 40 or 50 contiguous amino acids of the full-length sequence set forth in SEQ ID NO:2.

By "high stringency hybridization conditions" is meant those hybridizing conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinyl-pyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS. Under stringent hybridization conditions only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having 1 or 2 mismatches out of 20 contiguous nucleotides.

Methods for using the probes include detecting the presence or amount hRXR-γ RNA in a sample by contacting the sample with a nucleic acid probe under conditions such that hybridization occurs and detecting the presence or amount of the probe bound to hRXR-γ RNA. The nucleic acid duplex formed between the probe and a nucleic acid sequence coding for a hRXR-γ polypeptide may be used in the identification of the sequence of the nucleic acid detected (for example see, Nelson *et al.*, in Nonisotopic DNA Probe Techniques, p. 275 Academic Press, San Diego (Kricka, ed., 1992) hereby incorporated by reference herein in its entirety, including any drawings). Kits for performing such methods may be constructed to include a container means having disposed therein a nucleic acid probe.

The invention features recombinant nucleic acid comprising a contiguous nucleic acid sequence encoding a hRXR-γ polypeptide, preferably in a cell or an organism. The recombinant nucleic acid may contain a sequence set

forth in SEQ ID NO:1 and a vector or a promoter effective to initiate transcription in a host cell. The recombinant nucleic acid can alternatively contain a transcriptional initiation region functional in a cell, a sequence complimentary to an RNA sequence encoding a hRXR-γ polypeptide and a transcriptional termination region functional in a cell.

In another aspect the invention features an isolated, enriched, purified or recombinant hRXR-γ polypeptide.

By "isolated" in reference to a polypeptide is meant a polymer of 2 (preferably 7, more preferably 13, most preferably 25) or more amino acids conjugated to each other, including polypeptides that are isolated from a natural source or that are synthesized. The isolated polypeptides of the present invention are unique in the sense that they are not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only amino acid chain present, but that it is the predominate sequence present (at least 10 - 20% more than any other sequence) and is essentially free (about 90 - 95% pure at least) of non-amino acid material naturally associated with it.

By "enriched" in reference to a polypeptide is meant that the specific amino acid sequence constitutes a significantly higher fraction (2 - 5 fold) of the total of amino acids present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reduction in the amount of other amino acids present, or by a preferential increase in the amount of the specific amino acid sequence of interest, or by a combination of the two. However, it should be noted that enriched does not imply that there are no other amino

acid sequences present, just that the relative amount of the sequence of interest has been significantly increased. The term "significantly" here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other amino acids of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there is no amino acid from other sources. The amino acid from other sources may, for example, comprise amino acid encoded by a yeast or bacterial genome, or a cloning vector such as pUC19. The term is meant to cover only those situations in which man has intervened to elevate the proportion of the desired amino acid.

By "purified" in reference to a polypeptide does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, e.g., in terms of mg/ml). Purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. The substance is preferably free of contamination at a functionally significant level, for example 90%, 95%, or 99% pure.

By "recombinant hRXR-γ polypeptide" is meant a hRXR-γ polypeptide produced by recombinant DNA techniques such that it is distinct from a naturally occurring polypeptide either in its location (e.g., present in a different cell or tissue than found in nature), purity or structure. Generally, such a recombinant polypeptide will be present in a cell in an amount different from that normally observed in nature. This invention features recombinant hRXR-γ polypeptides obtainable using techniques known to those skilled in the art, including those described in McDonnell et al., PCT Publication No. WO 94/23068 published October 13, 1994, Evans et al., U.S. Patent

5,071,773, and PCT application, PCT/US91/00399 filed January 22, 1991 (International Publication No. WO 91/12258), incorporated by reference herein.

In a preferred embodiment, either vector pBacPAK8 (Clontech) or vector pBacPAK9 (Clontech) is used to express recombinant hRXR-γ polypeptide in insect cells. In another preferred embodiment, vector pYES2 (Invitrogen) is used to express recombinant hRXR-γ polypeptide in yeast cells. In yet another preferred embodiment, pBKCMV (Stratagene) is used to express recombinant hRXR-γ polypeptide in mammalian cells.

In preferred embodiments the hRXR-γ polypeptide contains at least 9, 10, 15, 20, or 30 contiguous amino acids of the full-length sequence set forth in SEQ ID NO:2.

In yet another aspect the invention features a purified antibody (e.g., a monoclonal or polyclonal antibody) having specific binding affinity to a hRXR-γ polypeptide. The antibody contains a sequence of amino acids that is able to specifically bind to a hRXR-γ polypeptide. An anti-peptide antibody may be prepared with techniques known to those skilled in the art, including, but not limited to, those disclosed in Niman, PCT application PCT/US88/03921 (International Publication No. WO 89/04489), incorporated by reference herein.

By "specific binding affinity" is meant that the antibody will bind to a hRXR-γ polypeptide at a certain detectable amount but will not bind other polypeptides to the same extent under identical conditions.

Antibodies having specific binding affinity to a hRXR-γ polypeptide may be used in methods for detecting the presence and/or amount of a hRXR-γ polypeptide in a sample by contacting the sample with the antibody under conditions such that an immunocomplex forms and detecting the presence and/or amount of the antibody conjugated to the hRXR-γ polypeptide. Diagnostic kits for performing such methods may be constructed to include a first

container means containing the antibody and a second container means having a conjugate of a binding partner of the antibody and a label.

In another aspect the invention features a hybridoma which produces an antibody having specific binding affinity to a hRXR-γ polypeptide.

By "hybridoma" is meant an immortalized cell line which is capable of secreting an antibody, for example a hRXR-γ antibody.

In preferred embodiments the hRXR-γ antibody comprises a sequence of amino acids that is able to specifically bind a hRXR-γ polypeptide.

In other aspects, the invention provides transgenic, nonhuman mammals containing a transgene encoding a hRXR-γ polypeptide or a gene effecting the expression of a hRXR-γ polypeptide. Such transgenic nonhuman mammals are particularly useful as an *in vivo* test system for studying the effects of introducing a hRXR-γ polypeptide, regulating the expression of a hRXR-γ polypeptide (i.e., through the introduction of additional genes, antisense nucleic acids, or ribozymes).

A "transgenic animal" is an animal having cells that contain DNA which has been artificially inserted into a cell, which DNA becomes part of the genome of the animal which develops from that cell. Preferred transgenic animals are primates, mice, rats, cows, pigs, horses, goats, sheep, dogs and cats. The transgenic DNA may encode for a hRXR-γ polypeptide. Native expression in an animal may be reduced by providing an amount of anti-sense RNA or DNA effective to reduce expression of the receptor.

In another aspect, the invention describes a recombinant cell or tissue containing a purified nucleic acid coding for a hRXR-γ polypeptide. In such cells, the nucleic acid may be under the control of its genomic regulatory elements, or may be under the control of exogenous regulatory elements including an exogenous promoter. By "exogenous" it is meant a promoter that is not normally

coupled *in vivo* transcriptionally to the coding sequence for the hRXR-γ polypeptide.

Another aspect of the invention features a method of detecting the presence or amount of a compound capable of binding to a hRXR-γ polypeptide. The method involves incubating the compound with a hRXR-γ polypeptide and detecting the presence or amount of the compound bound to the hRXR-γ polypeptide. The present invention also features novel compounds capable of binding hRXR-γ polypeptide that are identified by methods described above.

In a preferred embodiment, a cell or an *in vitro* system is transformed with a vector expressing hRXR-γ polypeptide and a reporter gene which becomes activated when a ligand binds to hRXR-γ polypeptide. Then said cell or *in vitro* system is brought into contact with a test compound. An increase in the activity of the reporter gene would indicate that the test compound is capable of binding hRXR-γ polypeptide.

In an example, the DNA-binding domain of hRXR-γ is replaced with the DNA-binding domain of a well characterized nuclear receptor, such as the glucocorticoid or estrogen receptor, to create a chimeric receptor able to activate a glucocorticoid- or estrogen-responsive reporter gene in the presence of the hRXR-γ-specific ligand (Giguere, V. and Evans, RM 1990, "Identification of receptors for retinoids as members of the steroid and thyroid hormone receptor family", In : Packer L (ed) Retinoids. Part A: Molecular and Metabolic Aspects. Methods in Enzymology. Academic Press, San Diego, CA, 189:223-232, incorporated by reference herein). The cell is transformed with the chimeric receptor. The cell is also transformed with a reporter vector which comprises a segment encoding a reporter polypeptide under the control of a promoter and a segment of hormone response element (such as a glucocorticoid- or estrogen-responsive element). When a suitable hormone or ligand is provided to the cell, a hormone receptor - hormone complex is

formed and delivered to an appropriate DNA-binding region to thereby activate the hormone response element and cause expression of the reporter gene. Activation of the reporter gene is detected by standard procedures used for detecting the product of the reporter gene. After introduction of the chimeric receptor and report gene constructs in recipient cells by transient transection, the cells are challenged with a battery of ligands until a positive response is observed.

The compounds identified by the method of this invention are particularly useful in the treatment of skin-related diseases, including, without limitation, actinic keratoses, axenic keratoses, inflammatory and non-inflammatory acne, psoriasis, ichthyoses and other keratinization and hyperproliferative disorders of the skin, eczema, atopic dermatitis, Darriers disease, lichen planus, prevention and reversal of glucocorticoid damage (steroid atrophy) as a topical anti-microbial, as skin pigmentation agents and to treat and reverse the effects of age and photo damage to the skin. The compounds are also useful for the prevention and treatment of cancerous and pre-cancerous conditions, including, premalignant and malignant hyperproliferative diseases such as cancers of the breast, skin, prostate, cervix, uterus, colon, bladder, esphoagus, stomach, lung, larynx, oral cavity, blood and lymphatic system, metaplasias, dysplasias, neoplasias, leukoplakias and papillomas of the mucous membranes and in the treatment of Kaposis sarcoma. In addition, the present compounds can be used as agents to treat diseases of the eye, including, without limitation, proliferative vitreoretinopathy (PVR), retinal detachment, dry eye and other corneopathies, as well as in the treatment and prevention of various cardiovascular dyslipidemias, prevention of restenosis and as an agent to increase the level of circulating tissue plasminogen activator (TPA). Other uses for the compounds of the present invention include the prevention and treatment of

conditions and diseases associated with Human papilloma virus (HPV), including warts and genital warts, various inflammatory diseases such as pulmonary fibrosis, ileitis, colitis and Krohn's disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Lou Gehrigs disease, improper pituitary function, including insufficient production of growth hormone, modulation of apoptosis, including both the induction of apoptosis and inhibition of T-Cell activated apoptosis, restoration of hair growth, including combination therapies with the present compounds and other agents such as Minosidil, diseases associated with the immune system, including use of the present compounds as immunosuppressants and immunostimulants, modulation of organ transplant rejection and facilitation of wound healing, including modulation of chelosis. The compounds identified herein may be used in combination with radiation therapy, chemotherapy and other biologicals such as interferons and interleukins.

The present invention also includes pharmaceutically acceptable compositions prepared for storage and subsequent administration which include a pharmaceutically effective amount of an above-described product in a pharmaceutically acceptable carrier or diluent.

By "therapeutically effective amount" is meant an amount of a pharmaceutical composition having a therapeutically relevant effect. A therapeutically relevant effect relieves to some extent one or more symptoms of the disease or condition in the patient; or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of the disease or condition.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

The present invention relates to hRXR-γ polypeptides, nucleic acids encoding such polypeptides, cells, tissues and animals containing such nucleic acids, antibodies to such polypeptides, assays utilizing such polypeptides, and methods relating to all of the foregoing.

I. Nucleic Acid Encoding A hRXR-γ Polypeptide.

Included within the scope of this invention are the functional equivalents of the herein-described isolated nucleic acid molecules. The degeneracy of the genetic code permits substitution of certain codons by other codons which specify the same amino acid and hence would give rise to the same protein. The nucleic acid sequence can vary substantially since, with the exception of methionine and tryptophan, the known amino acids can be coded for by more than one codon. Thus, portions or all of the hRXR-γ gene could be synthesized to give a nucleic acid sequence significantly different from that shown in SEQ ID NO: 1. The encoded amino acid sequence thereof would, however, be preserved.

In addition, the nucleic acid sequence may comprise a nucleotide sequence which results from the addition, deletion or substitution of at least one nucleotide to the 5'-end and/or the 3'-end of the nucleic acid formula shown in SEQ ID NO: 1 or a derivative thereof. Any nucleotide or polynucleotide may be used in this regard, provided that its addition, deletion or substitution does not alter the amino acid sequence of SEQ ID NO:2 which is encoded by the nucleotide sequence. For example, the present invention is intended to include any nucleic acid sequence resulting from the addition of ATG as an initiation codon at the 5'-end of the inventive nucleic acid sequence or its derivative, or from the addition of TTA, TAG or TGA as a termination codon at the 3'-end of the inventive nucleotide sequence or its derivative. Moreover, the nucleic acid molecule of the present invention may, as necessary,

have restriction endonuclease recognition sites added to its 5'-end and/or 3'-end.

Such functional alterations of a given nucleic acid sequence afford an opportunity to promote secretion and/or processing of heterologous proteins encoded by foreign nucleic acid sequences fused thereto. All variations of the nucleotide sequence of the hRXR-γ genes and fragments thereof permitted by the genetic code are, therefore, included in this invention.

Further, it is possible to delete codons or to substitute one or more codons by codons other than degenerate codons to produce a structurally modified polypeptide, but one which has substantially the same utility or activity of the polypeptide produced by the unmodified nucleic acid molecule. As recognized in the art, the two polypeptides are functionally equivalent, as are the two nucleic acid molecules which give rise to their production, even though the differences between the nucleic acid molecules are not related to degeneracy of the genetic code.

II. A Nucleic Acid Probe for the Detection of hRXR-γ.

A nucleic acid probe of the present invention may be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain another nucleic acid molecule of the present invention. A chromosomal DNA or cDNA library may be prepared from appropriate cells according to recognized methods in the art (cf. Molecular Cloning: A Laboratory Manual, second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

In the alternative, chemical synthesis is carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to N-terminal and C-terminal portions of the amino acid sequence of the polypeptide of interest. Thus, the synthesized nucleic acid probes may be used as primers in a polymerase chain reaction (PCR)

carried out in accordance with recognized PCR techniques, essentially according to PCR Protocols, A Guide to Methods and Applications, edited by Michael et al., Academic Press, 1990, utilizing the appropriate chromosomal or cDNA library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes based on the sequence disclosed herein using methods of computer alignment and sequence analysis known in the art (cf. Molecular Cloning: A Laboratory Manual, second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989). The hybridization probes of the present invention can be labeled by standard labeling techniques such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemi-luminescence, and the like. After hybridization, the probes may be visualized using known methods.

The nucleic acid probes of the present invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art. The nucleic acid probe may be immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins, such as polyacrylamide and latex beads. Techniques for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The sample used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample which is compatible with the method utilized.

III. Probe Based Method And Kit For Detecting hRXR-γ.

One method of detecting the presence of hRXR-γ in a sample comprises a) contacting said sample with the above-described nucleic acid probe, under conditions such that hybridization occurs, and b) detecting the presence of said probe bound to said nucleic acid molecule. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples of human tissue.

A kit for detecting the presence of hRXR-γ in a sample comprises at least one container means having disposed therein the above-described nucleic acid probe. The kit may further comprise other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or steptavidin).

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like. One skilled in the art will readily

recognize that the nucleic acid probes described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

IV. DNA Constructs Comprising a hRXR-γ Nucleic Acid Molecule and Cells Containing These Constructs.

The present invention also relates to a recombinant DNA molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described nucleic acid molecules. In addition, the present invention relates to a recombinant DNA molecule comprising a vector and an above-described nucleic acid molecules. The present invention also relates to a nucleic acid molecule comprising a transcriptional region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide, and a transcriptional termination region functional in said cell. The above-described molecules may be isolated and/or purified DNA molecules.

The present invention also relates to a cell or organism that contains an above-described nucleic acid molecule. The peptide may be purified from cells which have been altered to express the peptide. A cell is said to be "altered to express a desired peptide" when the cell, through genetic manipulation, is made to produce a protein which it normally does not produce or which the cell normally produces at lower levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode

the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression may vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the sequence encoding an hRXR-γ gene may be obtained by the above-described methods. This region may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding an hRXR-γ gene, the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted.

Two DNA sequences (such as a promoter region sequence and a hRXR-γ sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of a hRXR-γ gene sequence, or (3) interfere with the ability of a hRXR-γ gene sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence. Thus, to express a hRXR-γ gene, transcriptional and

translational signals recognized by an appropriate host are necessary.

The present invention encompasses the expression of the hRXR-γ gene (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, very efficient and convenient for the production of recombinant proteins and are, therefore, one type of preferred expression system for the hRXR-γ gene. Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains may also be used, including other bacterial strains.

In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host may be used. Examples of suitable plasmid vectors may include pBR322, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors may include λgt10, λgt11 and the like; and suitable virus vectors may include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.

Recognized prokaryotic hosts include bacteria such as E. coil, Bacillus, Streptomyces, Pseudomonas, Salmonella, Serratia, and the like. However, under such conditions, the peptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

To express hRXR-γ (or a functional derivative thereof) in a prokaryotic cell, it is necessary to operably link the hRXR-γ sequence to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the int promoter of bacteriophage λ, the bla promoter of the β-lactamase gene sequence of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene sequence of pPR325, and the like. Examples of inducible prokaryotic

promoters include the major right and left promoters of bacteriophage λ (P_L and P_R), the trp, recA, lacZ, lacI, and gal promoters of E. coli, the α-amylase (Ulmanen et at., J. Bacteriol. 162:176-182(1985)) and the ς-28-specific promoters of B. subtilis (Gilman et at., Gene sequence 32:11-20(1984)), the promoters of the bacteriophages of Bacillus (Gryczan, In: The Molecular Biology of the Bacilli, Academic Press, Inc., NY (1982)), and Streptomyces promoters (Ward et at., Mol. Gen. Genet. 203:468-478(1986)). Prokaryotic promoters are reviewed by Glick (J. Ind. Microbiot. 1:277-282(1987)); Cenatiempo (Biochimie 68:505-516(1986)); and Gottesman (Ann. Rev. Genet. 18:415-442 (1984)).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold et at. (Ann. Rev. Microbiol. 35:365-404(1981)). The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene. As used herein, "cell", "cell line", and "cell culture" may be used interchangeably and all such designations include progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant progeny have the same functionality as that of the originally transformed cell.

Host cells which may be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the hRXR-γ polypeptide of interest. Suitable hosts may often include eukaryotic cells. Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, mammalian cells either in vivo, or in tissue culture.

Mammalian cells which may be useful as hosts include HeLa cells, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives. Preferred mammalian host cells include SP2/0 and J558L, as well as neuroblastoma cell lines such as IMR 332 which may provide better capacities for correct post-translational processing.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences. Another preferred host is an insect cell, for example the Drosophila larvae. Using insect cells as hosts, the Drosophila alcohol dehydrogenase promoter can be used. Rubin, Science 240:1453-1459(1988). Alternatively, baculovirus vectors can be engineered to express large amounts of hRXR-γ in insects cells (Jasny, Science 238:1653 (1987); Miller et al., In: Genetic Engineering (1986), Setlow, J.K., et al., eds., Plenum, Vol. 8, pp. 277-297).

Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes are produced in large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals. Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes peptides bearing leader sequences (i.e., pre-peptides). For a mammalian host, several possible vector systems are available for the expression of hRXR-γ.

A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, cytomegalovirus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, and the like, may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical (such as metabolite) regulation.

Expression of hRXR-γ in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter of the mouse metallothionein I gene sequence (Hamer et al., J. Mol. Appl. Gen. 1:273-288(1982)); the TK promoter of Herpes virus (McKnight, Cell 31:355-365 (1982)); the SV40 early promoter (Benoist et al., Nature (London) 290:304-310(1981)); the yeast gal4 gene sequence promoter (Johnston et al., Proc. Natl. Acad. Sci. (USA) 79:6971-6975(1982); Silver et al., Proc. Natl. Acad. Sci. (USA) 81:5951-5955 (1984)).

Translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes hRXR-γ (or a functional derivative thereof) does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such

codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the hRXR-γ coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the hRXR-γ coding sequence).

A hRXR-γ nucleic acid molecule and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a nonreplicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene may occur through the transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced DNA sequence into the host chromosome.

A vector may be employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, Molec. Cell. Biol. 3:280(1983).

The introduced nucleic acid molecule can be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a

wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic vectors include plasmids such as those capable of replication in E. coil (such as, for example, pBR322, ColE1, pSC101, pACYC 184, πVX. Such plasmids are, for example, disclosed by Sambrook (cf. Molecular Cloning: A Laboratory Manual, second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, (1989)). Bacillus plasmids include pC194, pC221, pT127, and the like. Such plasmids are disclosed by Gryczan (In: The Molecular Biology of the Bacitli, Academic Press, NY (1982), pp. 307-329). Suitable Streptomyces plasmids include p1J101 (Kendall et al., J. Bacteriol. 169:4177-4183 (1987)), and streptomyces bacteriophages such as ϕC31 (Chater et al., In: Sixth International Symposium on Actinomycetales Biology, Akademiai Kaido, Budapest, Hungary (1986), pp. 45-54). Pseudomonas plasmids are reviewed by John et al. (Rev. Infect. Dis. 8:693-704(1986)), and Izaki (Jpn. J. Bacteriol. 33:729-742(1978)).

Preferred eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known in the art (Botstein et al., Miami Wntr. Symp. 19:265-274(1982); Broach, In: The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, Cell 28:203-204 (1982); Bollon et at., J. Ctin. Hematol. Oncol. 10:39-48 (1980); Maniatis, In: Cell Biology: A Comprehensive Treatise, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608(1980).

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, i.e., transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the production of hRXR-γ or fragments thereof. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like). A variety of incubation conditions can be used to form the peptide of the present invention. The most preferred conditions are those which mimic physiological conditions.

V. Purified hRXR-γ Polypeptides.

A variety of methodologies known in the art can be utilized to obtain the peptide of the present invention. The peptide may be purified from tissues or cells which naturally produce the peptide. Alternatively, the above-described isolated nucleic acid fragments could be used to expressed the hRXR-γ protein in any organism. The samples of the present invention include cells, protein extracts or membrane extracts of cells, or biological fluids. The sample will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts used as the sample.

Any eukaryotic organism can be used as a source for the peptide of the invention, as long as the source organism naturally contains such a peptide. As used herein, "source organism" refers to the original organism from which the amino acid sequence of the subunit is derived,

regardless of the organism the subunit is expressed in and ultimately isolated from.

One skilled in the art can readily follow known methods for isolating proteins in order to obtain the peptide free of natural contaminants. These include, but are not limited to: size-exclusion chromatography, HPLC, ion-exchange chromatography, and immuno-affinity chromatography.

VI. hRXR-γ Antibody And Hybridoma.

The present invention relates to an antibody having binding affinity to a hRXR-γ polypeptide. The polypeptide may have the amino acid sequence set forth in SEQ ID NO:2, or mutant or species variation thereof, or at least 9 contiguous amino acids thereof (preferably, at least 10, 15, 20, or 30 contiguous amino acids thereof).

The present invention also relates to an antibody having specific binding affinity to an hRXR-γ polypeptide. Such an antibody may be isolated by comparing its binding affinity to a hRXR-γ polypeptide with its binding affinity to another polypeptide. Those which bind selectively to hRXR-γ would be chosen for use in methods requiring a distinction between hRXR-γ and other polypeptides.

The hRXR-γ proteins of the present invention can be used in a variety of procedures and methods, such as for the generation of antibodies, for use in identifying pharmaceutical compositions, and for studying DNA/protein interaction.

The hRXR-γ peptide of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide would be generated as described herein and used as an immunogen. The antibodies of the present invention include monoclonal and polyclonal antibodies, as well fragments of these antibodies, and humanized forms. Humanized forms of the antibodies of the present invention may be generated using one of the procedures known in the

art such as chimerization or CDR grafting. The present invention also relates to a hybridoma which produces the above-described monoclonal antibody, or binding fragment thereof. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth et al., J. Immunol. Methods 35:1-21(1980)). Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or intraperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal which is immunized, the antigenicity of the polypeptide and the site of injection.

The polypeptide may be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or β-galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz et al., Exp. Cell Res. 175:109-124(1988)). Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using

procedures known in the art (Campbell, Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology, supra (1984)).

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures. The above-described antibodies may be detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such as horse radish peroxidase, alkaline phosphatase, and the like) fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, see (Stemberger et al., J. Histochem. Cytochem. 18:315(1970); Bayer et at., Meth. Enzym. 62:308(1979); Engval et al., Immunot. 109:129(1972); Goding, J. Immunol. Meth. 13:215(1976)). The labeled antibodies of the present invention can be used for in vitro, in vivo, and in situ assays to identify cells or tissues which express a specific peptide.

The above-described antibodies may also be immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir et al., "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10(1986); Jacoby et al., Meth. Enzym. 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for in vitro, in vivo, and in situ assays as well as in immunochromotography.

Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques,

methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby et al., "Application of Synthetic Peptides: Antisense Peptides", In Synthetic Peptides, A User's Guide, W.H. Freeman, NY, pp. 289-307(1992), and Kaspczak et al., Biochemistry 28:9230-8(1989).

Anti-peptide peptides can be generated by replacing the basic amino acid residues found in the hRXR-γ peptide sequence with acidic residues, while maintaining hydrophobic and uncharged polar groups. For example, lysine, arginine, and/or histidine residues are replaced with aspartic acid or glutamic acid and glutamic acid residues are replaced by lysine, arginine or histidine.

VII. An Antibody Based Method And Kit For Detecting hRXR-γ.

The present invention encompasses a method of detecting a hRXR-γ polypeptide in a sample, comprising: a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample.

Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available immunological assay formats (such as radioimmunoassays, enzyme-linked immunosorbent assays, diffusion based Ouchterlony, or rocket immunofluorescent assays) can readily be adapted to employ the antibodies of the present invention. Examples of such assays can be

found in Chard, "An Introduction to Radioimmunoassay and Related Techniques" Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock et al., "Techniques in Immunocytochemistry," Academic Press, Orlando, FL Vol. 1(1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, "Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is capable with the system utilized.

A kit contains all the necessary reagents to carry out the previously described methods of detection. The kit may comprise: i) a first container means containing an above-described antibody, and ii) second container means containing a conjugate comprising a binding partner of the antibody and a label. In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound antibodies.

Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. The compartmentalized kit may be as described above for nucleic acid probe kits. One skilled in the art will readily recognize that the antibodies described in the present

invention can readily be incorporated into one of the established kit formats which are well known in the art.

VIII. Isolation of Compounds Which Interact With hRXR-γ.

The present invention also relates to a method of detecting a compound capable of binding to a hRXR-γ polypeptide comprising incubating the compound with hRXR-γ and detecting the presence of the compound bound to hRXR-γ. The compound may be present within a complex mixture, for example, serum, body fluid, or cell extracts.

The present invention also relates to a method of detecting an agonist or antagonist of hRXR-γ activity comprising incubating cells that produce hRXR-γ in the presence of a compound and detecting changes in the level of hRXR-γ activity. The compounds thus identified would produce a change in activity indicative of the presence of the compound. The compound may be present within a complex mixture, for example, serum, body fluid, or cell extracts. Once the compound is identified it can be isolated using techniques well known in the art.

The present invention also encompasses a method of agonizing (stimulating) or antagonizing hRXR-γ associated activity in a mammal comprising administering to said mammal an agonist or antagonist to hRXR-γ in an amount sufficient to effect said agonism or antagonism.

IX. Transgenic Animals.

A variety of methods are available for the production of transgenic animals associated with this invention. DNA can be injected into the pronucleus of a fertilized egg before fusion of the male and female pronuclei, or injected into the nucleus of an embryonic cell (e.g., the nucleus of a two-cell embryo) following the initiation of cell division (Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985)). Embryos can be infected with viruses, especially retroviruses, modified to carry

inorganic-ion receptor nucleotide sequences of the invention.

Pluripotent stem cells derived from the inner cell mass of the embryo and stabilized in culture can be manipulated in culture to incorporate nucleotide sequences of the invention. A transgenic animal can be produced from such cells through implantation into a blastocyst that is implanted into a foster mother and allowed to come to term. Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Charles River (Wilmington, MA), Taconic (Germantown, NY), Harlan Sprague Dawley (Indianapolis, IN), etc.

The procedures for manipulation of the rodent embryo and for microinjection of DNA into the pronucleus of the zygote are well known to those of ordinary skill in the art (Hogan et al., supra). Microinjection procedures for fish, amphibian eggs and birds are detailed in Houdebine and Chourrout, Experientia 47: 897-905 (1991). Other procedures for introduction of DNA into tissues of animals are described in U.S. Patent No., 4,945,050 (Sandford et al., July 30, 1990).

By way of example only, to prepare a transgenic mouse, female mice are induced to superovulate. Females are placed with males, and the mated females are sacrificed by CO_2 asphyxiation or cervical dislocation and embryos are recovered from excised oviducts. Surrounding cumulus cells are removed. Pronuclear embryos are then washed and stored until the time of injection. Randomly cycling adult female mice are paired with vasectomized males. Recipient females are mated at the same time as donor females. Embryos then are transferred surgically. The procedure for generating transgenic rats is similar to that of mice. See Hammer et al., Cell 63:1099-1112 (1990).

Methods for the culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods

such as electroporation, calcium phosphate/DNA precipitation and direct injection also are well known to those of ordinary skill in the art. See, for example, Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E.J. Robertson, ed., IRL Press (1987).

In cases involving random gene integration, a clone containing the sequence(s) of the invention is co-transfected with a gene encoding resistance. Alternatively, the gene encoding neomycin resistance is physically linked to the sequence(s) of the invention. Transfection and isolation of desired clones are carried out by any one of several methods well known to those of ordinary skill in the art (E.J. Robertson, supra).

DNA molecules introduced into ES cells can also be integrated into the chromosome through the process of homologous recombination. Capecchi, Science 244: 1288-1292 (1989). Methods for positive selection of the recombination event (i.e., neo resistance) and dual positive-negative selection (i.e., neo resistance and gancyclovir resistance) and the subsequent identification of the desired clones by PCR have been described by Capecchi, supra and Joyner et al., Nature 338: 153-156 (1989), the teachings of which are incorporated herein. The final phase of the procedure is to inject targeted ES cells into blastocysts and to transfer the blastocysts into pseudopregnant females. The resulting chimeric animals are bred and the offspring are analyzed by Southern blotting to identify individuals that carry the transgene. Procedures for the production of non-rodent mammals and other animals have been discussed by others. See Houdebine and Chourrout, supra; Pursel et al., Science 244:1281-1288 (1989); and Simms et al., Bio/Technology 6:179-183 (1988).

X. Gene Therapy

hRXRγ or its genetic sequences will be useful in gene therapy (reviewed in Miller, *Nature* 357:455-460, (1992).

Miller states that advances have resulted in practical approaches to human gene therapy that have demonstrated positive initial results. An *in vivo* model of gene therapy for human severe combined immunodeficiency is described in Ferrari, et al., *Science* 251:1363-1366, (1991). The basic science of gene therapy is described in Mulligan, *Science* 260:926-931, (1993).

In one preferred embodiment, an expression vector containing the hRXRγ coding sequence is inserted into cells, the cells are grown *in vitro* and then infused in large numbers into patients. In another preferred embodiment, a DNA segment containing a promoter of choice (for example a strong promoter) is transferred into cells containing an endogenous hRXRγ in such a manner that the promoter segment enhances expression of the endogenous hRXRγ gene (for example, the promoter segment is transferred to the cell such that it becomes directly linked to the endogenous hRXRγ gene).

The gene therapy may involve the use of an adenovirus containing hRXRγ cDNA targeted to a tumor, systemic hRXRγ increase by implantation of engineered cells, injection with hRXRγ virus, or injection of naked hRXRγ DNA into appropriate tissues.

Target cell populations (e.g., hematopoietic or nerve cells) may be modified by introducing altered forms of hRXRγ in order to modulate the activity of such cells.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adenovirus, adeno-associated virus, herpes viruses, several RNA viruses, or bovine papilloma virus, may be used for delivery of nucleotide sequences (e.g., cDNA) encoding recombinant hRXRγ protein into the targeted cell population (e.g., tumor cells). Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors containing coding sequences. See, for example, the techniques described in Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y.

(1989), and in Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. (1989). Alternatively, recombinant nucleic acid molecules encoding protein sequences can be used as naked DNA or in reconstituted system e.g., liposomes or other lipid systems for delivery to target cells (See e.g., Felgner et al., *Nature* 337:387-8, 1989). Several other methods for the direct transfer of plasmid DNA into cells exist for use in human gene therapy and involve targeting the DNA to receptors on cells by complexing the plasmid DNA to proteins. See, Miller, supra.

In its simplest form, gene transfer can be performed by simply injecting minute amounts of DNA into the nucleus of a cell, through a process of microinjection. Capecchi MR, Cell 22:479-88 (1980). Once recombinant genes are introduced into a cell, they can be recognized by the cells normal mechanisms for transcription and translation, and a gene product will be expressed. Other methods have also been attempted for introducing DNA into larger numbers of cells. These methods include: transfection, wherein DNA is precipitated with $CaPO_4$ and taken into cells by pinocytosis (Chen C. and Okayama H, Mol. Cell Biol. 7:2745-52 (1987)); electroporation, wherein cells are exposed to large voltage pulses to introduce holes into the membrane (Chu G. et al., Nucleic Acids Res., 15:1311-26 (1987)); lipofection/liposome fusion, wherein DNA is packaged into lipophilic vesicles which fuse with a target cell (Felgner PL., et al., Proc. Natl. Acad. Sci. USA. 84:7413-7 (1987)); and particle bombardment using DNA bound to small projectiles (Yang NS. et al., Proc. Natl. Acad. Sci. 87:9568-72 (1990)). Another method for introducing DNA into cells is to couple the DNA to chemically modified proteins.

It has also been shown that adenovirus proteins are capable of destabilizing endosomes and enhancing the uptake of DNA into cells. The admixture of adenovirus to

solutions containing DNA complexes, or the binding of DNA to polylysine covalently attached to adenovirus using protein crosslinking agents substantially improves the uptake and expression of the recombinant gene. Curiel DT et al., Am. J. Respir. Cell. Mol. Biol., 6:247-52 (1992).

As used herein "gene transfer" means the process of introducing a foreign nucleic acid molecule into a cell. Gene transfer is commonly performed to enable the expression of a particular product encoded by the gene. The product may include a protein, polypeptide, anti-sense DNA or RNA, or enzymatically active RNA. Gene transfer can be performed in cultured cells or by direct administration into animals. Generally gene transfer involves the process of nucleic acid contact with a target cell by non-specific or receptor mediated interactions, uptake of nucleic acid into the cell through the membrane or by endocytosis, and release of nucleic acid into the cytoplasm from the plasma membrane or endosome. Expression may require, in addition, movement of the nucleic acid into the nucleus of the cell and binding to appropriate nuclear factors for transcription.

As used herein "gene therapy" is a form of gene transfer and is included within the definition of gene transfer as used herein and specifically refers to gene transfer to express a therapeutic product from a cell *in vivo* or *in vitro*. Gene transfer can be performed *ex vivo* on cells which are then transplanted into a patient, or can be performed by direct administration of the nucleic acid or nucleic acid-protein complex into the patient.

In another preferred embodiment, a vector having nucleic acid sequences encoding hRXRγ is provided in which the nucleic acid sequence is expressed only in specific tissue. Methods of achieving tissue-specific gene expression as set forth in International Publication No. WO 93/09236, filed November 3, 1992 and published May 13, 1993.

In all of the preceding vectors set forth above, a further aspect of the invention is that the nucleic acid sequence contained in the vector may include additions, deletions or modifications to some or all of the sequence of the nucleic acid, as defined above.

In another preferred embodiment, a method of gene replacement is set forth. "Gene replacement" as used herein means supplying a nucleic acid sequence which is capable of being expressed *in vivo* in an animal and thereby providing or augmenting the function of an endogenous gene which is missing or defective in the animal.

XI. Pharmaceutical Formulations and Modes of Administration

The particular compound or antibody that affects the disorder of interest can be administered to a patient either by themselves, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of a agent or agents such as these is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of

circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal disruption of the protein complex, or a half-maximal inhibition of the cellular level and/or activity of a complex component). Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl et al., in The Pharmacological Basis of Therapeutics, 1975, Ch. 1 p. 1). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the oncogenic disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents

may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may

contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropyl-methyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, option-

ally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Some methods of delivery that may be used include:

a. encapsulation in liposomes,

b. transduction by retroviral vectors,

c. localization to nuclear compartment utilizing nuclear targeting site found on most nuclear proteins,

d. transfection of cells *ex vivo* with subsequent reimplantation or administration of the transfected cells,

e. a DNA transporter system.

A hRXRγ nucleic acid sequence may be administered utilizing an *ex vivo* approach whereby cells are removed from an animal, transduced with the hRXRγ nucleic acid sequence and reimplanted into the animal. The liver can be accessed by an *ex vivo* approach by removing hepatocytes from an animal, transducing the hepatocytes *in vitro* with the hRXRγ nucleic acid sequence and reimplanting them into the animal (*e.g.*, as described for rabbits by Chowdhury et al, Science 254: 1802-1805, 1991, or in humans by Wilson, Hum. Gene Ther. 3: 179-222, 1992) incorporated herein by reference.

Many nonviral techniques for the delivery of a hRXRγ nucleic acid sequence into a cell can be used, including direct naked DNA uptake (*e.g.*, Wolff et al., Science 247: 1465-1468, 1990), receptor-mediated DNA uptake, *e.g.*, using DNA coupled to asialoorosomucoid which is taken up by the asialoglycoprotein receptor in the liver (Wu and Wu, J. Biol. Chem. 262: 4429-4432, 1987; Wu et al., J. Biol. Chem. 266: 14338-14342, 1991), and liposome-mediated delivery (*e.g.*, Kaneda et al., Expt. Cell Res. 173: 56-69, 1987; Kaneda et al., Science 243: 375-378, 1989; Zhu et al., Science 261: 209-211, 1993). Many of these physical methods can be combined with one another and with viral

techniques; enhancement of receptor-mediated DNA uptake can be effected, for example, by combining its use with adenovirus (Curiel et al., Proc. Natl. Acad. Sci. USA 88: 8850-8854, 1991; Cristiano et al., Proc. Natl. Acad. Sci. USA 90: 2122-2126, 1993).

The hRXRγ or nucleic acid encoding hRXRγ may also be administered via an implanted device that provides a support for growing cells. Thus, the cells may remain in the implanted device and still provide the useful and therapeutic agents of the present invention.

Examples

The examples below are non-limiting and are merely representative of various aspects and features of the procedures used to identify the full-length nucleic acid and amino acid sequences of hRXR-γ.

cDNA cloning

What follows is an example of the cloning of a hRXR-γ from a human heart cDNA library. Those of ordinary skill in the art will recognize that equivalent procedures can be readily used to isolate hRXR-γ from cDNA libraries or genomic libraries of other tissues.

The recipes for buffers, mediums, and solutions in the following experiments are given in J. Sambrook, E. F. Fritsch, and T. Maniatis, Molecular Cloning: A Laboratory Manual, 2 Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.

A human heart cDNA library, Human Heart 5'-STRETCH in λ-gt10, was purchased from Clontech Laboratories Inc., Palo Alto, California. A 1.7kb fragment isolated from a mouse RXR-γ cDNA clone (pSKmRXR-γ, (Mangelsdorf,D.J., Borgmeyer,U., Heyman,R.A., Zhou,J.Y., Ong,E.S., Oro,A.E., Kakizuka,A. and Evans,R.M. (1992) Genes Dev., 6, 329-344)) by digestion with EcoRI, was labeled with [^{32}P]-dCTP by random priming and was utilized to identify potential human RXR-γ cDNA clones.

Approximately $1.5x10^6$ phage plaques from the human heart cDNA library were screened with the mouse RXR-γ probe. The primary screen yielded 27 positively hybridizing phage plaques. Upon isolation and plaque purification, 23 phage plaques continued to give a strong hybridization signal. Each positively hybridizing phage plaque was purified to homogeneity and the inserts were amplified by PCR utilizing λ-gt10 primers containing internal BamHI sites.

PCR amplification of the 23 phage clones showed that 15 phages contained fragments greater than 500bp. These fragments fell into 9 different size groups ranging from 600bp to 1600bp. To ensure the fragments amplified by PCR were the phage clone fragments that hybridized to mouse RXR-γ, Southern blot analysis was performed on the amplified inserts; all amplified fragments continued to hybridize with the mouse RXR-γ probe.

To verify their identity, one each of the different size classes of PCR amplified fragments was subcloned into pGEM-4Z (Promega, Madison, WI) by digesting the amplified cDNA fragments with BamHI; the nucleotide sequence of the ends of each clone was determined.

Nucleotide sequence alignments showed that two of the clones (clone 10 and clone 27) were related to mouse RXR-γ; whereas three clones were human RXR-α and one clone was human RXR-β. After identifying the phage isolates that carried the human RXR-γ sequences, phage DNA was prepared and the inserts were subcloned from the phage DNA into pGEM-4Z. The size of each insert was 900bp for clone 10 and 1600bp for clone 27.

The nucleotide sequence of the complete human RXR-γ cDNA was determined by sequencing fragments isolated by restriction digestion and subcloning into pGEM-4Z; both strands of the clone were sequenced. All nucleotide sequencing was performed with the Automated Laser Fluorescence (A.L.F.) DNA Sequencer (Pharmacia Biotechnology, Piscataway, NJ). The entire nucleotide sequence was

compiled utilizing the Genetics Computer Group Sequence Analysis Software Package (Devereux,J., Haeberli,P. and Smithies,O. (1984) Nucleic. Acids. Res., 12, 387-395).

The complete nucleotide sequences of both clone 10 and clone 27 were determined. A comparison of the sequences indicated that clone 10 and clone 27 shared a 300bp overlap and the nucleotide sequence of both clones was identical in this region. Analysis of these data and comparison to mouse RXR-γ indicated that clone 27 contained the complete coding region and the clone terminated 23bp downstream of the termination codon. Clone 10 contained a portion of the coding region and the complete 3' untranslated region. The sequence shared between clone 10 and clone 27 contained a unique PstI site.

To construct a full-length clone, sequences 5' to the PstI site in clone 10 were replaced with the corresponding sequences from clone 27. This process generated a clone containing 1594 nucleotides. Sequence analysis indicates that the clone begins 28bp upstream of the initiator codon ATG and contains an in frame stop codon 12bp upstream of the ATG. The clone contains an open reading frame of 1389nt, a short 3' untranslated region containing a poly A addition site (AATAAA) followed 14nt later by a string of 22 adenine residues. There is a 89.3% nucleotide identity (i.e., "homology") between the human RXR-γ clone and the mouse RXR-γ sequence.

The deduced amino acid sequence of human RXR-γ predicts a protein of 463 amino acids. A comparison of the amino acid sequences between human and mouse show 98% amino acid sequence identity (i.e., "homology"). *In vitro* transcription followed by *in vitro* translation yields a protein product that has an apparent molecular mass of 55Kd when analyzed by SDS-polyacrylamide gel electrophoresis.

Expression Pattern of Human RXR-γ

To determine the expression pattern of human RXR-γ, applicant conducted Northern blot analysis of mRNAs isolated from various human tissues.

For mammalian expression studies, the entire human RXR-γ cDNA was subcloned into the EcoRI site of pcDNA-1 (Invitrogen, San Diego, CA) under the control of the CMV promoter.

A human multiple tissue Northern blot (Clontech Laboratories Inc.) containing 10μg of poly-A plus mRNA isolated from several human tissues was hybridized with the full length human RXR-γ cDNA that was random prime labeled with [^{32}P]-dCTP. The hybridization and all washes were conducted under high-stringency.

A message of approximately 2.2kb that specifically hybridizes with the human RXR-γ probe is found in high amounts in skeletal muscle and in lower amounts in heart, brain, lung and liver. A smaller message of 1.24kb is also seen in human liver. In addition to the 2.2kb and the 1.24kb transcripts, there is a third transcript of 6.5kb seen in kidney, skeletal muscle, liver, lung and placenta.

There are several notable differences between the expression pattern in humans and that in mouse. First, mouse tissues contain predominately two RXR-γ transcripts of 2.5kb and 2.0kb (Mangelsdorf,D.J., Borgmeyer,U., Heyman,R.A., Zhou,J.Y., Ong,E.S., Oro,A.E., Kakizuka,A. and Evans,R.M. (1992) Genes Dev., 6, 329-344); whereas human tissues contain three RXR-γ transcripts of 6.5kb, 2.2kb and 1.24kb.

Second, the different expression patterns seen in mouse between the 2.5kb and the 2.0kb fragment are not seen in human tissues. In the adult mouse the 2.5kb transcript is seen in brain and lung, the 2.0kb transcript is present in kidney and liver and both transcripts are expressed in mouse heart and muscle. There is no evidence that a similar expression pattern exists in human tissues.

The predominate transcript detected in human tissues is 2.2kb with the notable exception that this transcript is absent or undetectable in human liver. Human liver mRNA contains only the 1.24kb transcript. It is not clear whether the 1.24kb transcript is able to encode the entire open reading frame for RXR-γ, since this transcript (depending upon the accuracy of the size determination) is approximately 200nt shorter than the 1389nt needed to encode the entire RXR-γ protein. Only cloning and characterization of the 1.24kb transcript will clarify its identity and role in the human liver.

Ribonuclease Protection Assay

To utilize the human RXR-γ gene as a diagnostic tool to monitor the expression pattern of this retinoid receptor, applicant has developed a sensitive Ribonuclease protection assay from a fragment of this clone. The assay is as follows:

The RNA-protection probe for the human RXR-γ isoform was constructed by PCR amplification from the full length hRXR-γ cDNA clone; a 159bp fragment corresponding to nucleotides 1395-1553 of human RXR-γ was amplified. The resulting fragment was cloned into pGEM4Z, and its identity was confirmed by nucleotide sequencing. The specificity of this probe for human sequences was verified by testing the probe against both mouse, rat and human RNAs known to contain the corresponding mRNA. The cRNA probe was produced by linearizing the plasmid with EcoRI and followed by in vitro transcription in the presence of [^{32}P]-UTP utilizing T7 polymerase. A probe corresponding to human GAPDH was purchased from Ambion Inc and is utilized in all assays to control for RNA content in the sample.

Total cytoplasmic RNA was isolated as described (Favaloro et. al., 1981). RNA-protection analysis was performed as described (Zinn et. al., 1984; Melton et. al., 1984). Hybridization of cRNA probes was carried out

at 45°C overnight, followed by the addition of 300μl of RNase digestion buffer containing 40μg/ml of RNase A and 700 U/ml of RNase T1. RNase digestion was performed at 25°C for 1 hour. The RNase resistant fragments were resolved by electrophoresis on 6% urea-polyacrylamide sequencing gels. To control for RNA loading, a GAPDH probe was included in all samples. As approximate size markers [^{32}P]-labeled MspI digested fragments of pBR322 were run on all gels.

Using this probe to analyze the expression of RXR-γ in a number of different human cell lines, applicant has found expression of hRXR-γ gene in 2 of 7 human head and neck squamous cell carcinoma samples tested (TAC and WWL). hRXR-γ is not expressed in two hematopoietic cell lines, HL-60 and RPMI 8226, in a cervical carcinoma cell line, ME-180, in a breast cancer cell line, MCF-7, or in a prostate carcinoma cell line, LNCaP.

Co-transfection Assay

To determine whether the hRXR-γ cDNA clone produced a protein that was transcriptionally competent, applicant used a transient co-transfection assay with a reporter construct specific for the RXR class of retinoid receptors (CRBP(2)-tk-LUC) (Mangelsdorf,D.J., Umesono,K., Kliewer,S.A., Borgmeyer,U., Ong,E.S. and Evans,R.M. (1991) Cell, **66**, 555-561). A vector expressing human RXR-γ is transfected into CV-1 cells along with a reporter for monitoring RXR-activity and the CV-1 cells are exposed to retinoid specific compounds.

Co-transfection assays were performed exactly as previously described (Heyman,R.A., Mangelsdorf,D.J., Dyck,J.A., Stein,R.B., Eichele,G., Evans,R.M. and Thaller,C. (1992) Cell, **68**, 397-406, Allegretto,E.A., McClurg,M.R., Lazarchik,S.B., Clemm,D.L., Kerner,S.A., Elgort,M.G., Boehm,M.F., White,S.K., Pike,J.W. and Heyman,R.A. (1993) J. Biol. Chem., **268**, 26625-26633). Briefly, CV-1 cells were transiently transfected with a

mixture of plasmids including the hRXR-γ expression vector, a reporter plasmid containing two copies of the RXR response element from the cellular retinoic acid binding protein ($CRBP_{(2)}$-tk-LUC) and an expression vector for β-galactosidase. Each data point from the co-transfection assay was normalized to the β-galactosidase expression and is the average of 6 determinations (the CV was less the 15% in all cases).

When CV-1 cells are co-transfected as described above and treated with increasing concentrations of LG100069, i.e., (*E*)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid (Boehm,M.F., et al. (1994) J. Med. Chem., **37**, 2930-2941.), or 9-*cis* retinoic acid (a retinoid pan-agonist), there is a dose dependent increase in reporter activity. This data demonstrates that both 9-cis RA and LG100069 are efficient at activating the transcriptional properties of human RXR-γ.

Using such assays, one can readily screen for desired human retinoid X receptor isoform specific ligands.

All publications referenced are incorporated by reference herein, including the nucleic acid sequences and amino acid sequences listed in each publication.

Other embodiments are within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Ligand Pharmaceuticals Incorporated

(ii) TITLE OF INVENTION: HUMAN RETINOID X RECEPTOR γ

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

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(B) STREET: 633 West Fifth Street, Suite 4700

(C) CITY: Los Angeles

(D) STATE: California

(E) COUNTRY: USA

(F) POSTAL CODE (ZIP): 90071-2066

(G) TELEPHONE: (213) 489-1600

(H) TELEFAX: (213) 955-0440

(I) TELEX: 67-3510

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1594 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAGAGGAACA TGAACTGACG AGTAAACATG TATGGAAATT 40

ATTCTCACTT CATGAAGTTT CCCGCAGGCT ATGGAGGCTC 80

CCCTGGCCAC ACTGGCTCTA CATCCATGAG CCCATCAGCA 120

GCCTTGTCCA CAGGGAAGCC AATGGACAGC CACCCCAGCT 160

ACACAGATAC CCCAGTGAGT GCCCCACGGA CTCTGAGTGC 200
AGTGGGGACC CCCCTCAATG CCCTGGGCTC TCCATATCGA 240
GTCATCACCT CTGCCATGGG CCCACCCTCA GGAGCACTTG 280
CAGCGCCTCC AGGAATCAAC TTGGTTGCCC CACCCAGCTC 320
TCAGCTAAAT GTGGTCAACA GTGTCAGCAG TTCAGAGGAC 360
ATCAAGCCCT TACCAGGGCT TCCCGGGATT GGAAACATGA 400
ACTACCCATC CACCAGCCCC GGATCTCTGG TTAAACACAT 440
CTGTGCTATC TGTGGAGACA GATCCTCAGG AAAGCACTAC 480
GGGGTATACA GTTGTGAAGG CTGCAAAGGG TTCTTCAAGA 520
GGACGATAAG GAAGGACCTC ATCTACACGT GTCGGGATAA 560
TAAAGACTGC CTCATTGACA AGCGTCAGCG CAACCGCTGC 600
CAGTACTGTC GCTATCAGAA GTGCCTTGTC ATGGGCATGA 640
AGAGGGAAGC TGTGCAAGAA GAAAGACAGA GGAGCCGAGA 680
GCGAGCTGAG AGTGAGGCAG AATGTGCTAC CAGTGGTCAT 720
GAAGACATGC CTGTGGAGAG GATTCTAGAA GCTGAACTTG 760
CTGTTGAACC AAAGACAGAA TCCTATGGTG ACATGAATAT 800
GGAGAACTCG ACAAATGACC CTGTTACCAA CATATGTCAT 840
GCTGCTGACA AGCAGCTTTT CACCCTCGTT GAATGGGCCA 880
AGCGTATTCC CCACTTCTCT GACCTCACCT TGGAGGACCA 920
GGTCATTTTG CTTCGGGCAG GGTGGAATGA ATTGCTGATT 960
GCCTCTTTCT CCCACCGCTC AGTTTCCGTG CAGGATGGCA 1000
TCCTTCTGGC CACGGGTTTA CATGTCCACC GGAGCAGTGC 1040
CCACAGTGCT GGGGTCGGCT CCATCTTTGA CAGAGTTCTA 1080
ACTGAGCTGG TTTCCAAAAT GAAAGACATG CAGATGGACA 1120
AGTCGGAACT GGGATGCCTG CGAGCCATTG TACTCTTTAA 1160
CCCAGATGCC AAGGGCCTGT CCAACCCCTC TGAGGTGGAG 1200
ACTCTGCGAG AGAAGGTTTA TGCCACCCTT GAGGCCTACA 1240
CCAAGCAGAA GTATCCGGAA CAGCCAGGCA GGTTTGCCAA 1280
GCTGCTGCTG CGCCTCCCAG CTCTGCGTTC CATTGGCTTG 1320
AAATGCCTGG AGCACCTCTT CTTCTTCAAG CTCATCGGGG 1360
ACACCCCCAT TGACACCTTC CTCATGGAGA TGTTGGAGAC 1400
CCCGCTGCAG ATCACCTGAG CCCCACCAGC CACAGCCTCC 1440
CCACCCAGGA TGACCCCTGG GCAGGTGTGT GTGGACCCCC 1480
ACCCTGCACT TTCCTCCACC TCCCACCCTG ACCCCCTTCC 1520
TGTCCCCAAA ATGTGATGCT TATAATAAAG AAAACCTTTC 1560
TACAAAAAAA AAAAAAAAAA AAAAACCGGA ATTC 1594

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 463 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Tyr Gly Asn Tyr Ser His Phe Met Lys Phe Pro Ala Gly
1 5 10

Tyr Gly Gly Ser Pro Gly His Thr Gly Ser Thr Ser Met Ser
15 20 25

Pro Ser Ala Ala Leu Ser Thr Gly Lys Pro Met Asp Ser His
30 35 40

Pro Ser Tyr Thr Asp Thr Pro Val Ser Ala Pro Arg Thr Leu
45 50 55

Ser Ala Val Gly Thr Pro Leu Asn Ala Leu Gly Ser Pro Tyr
60 65 70

Arg Val Ile Thr Ser Ala Met Gly Pro Pro Ser Gly Ala Leu
75 80

Ala Ala Pro Pro Gly Ile Asn Leu Val Ala Pro Pro Ser Ser
85 90 95

Gln Leu Asn Val Val Asn Ser Val Ser Ser Ser Glu Asp Ile
100 105 110

Lys Pro Leu Pro Gly Leu Pro Gly Ile Gly Asn Met Asn Tyr
115 120 125

Pro Ser Thr Ser Pro Gly Ser Leu Val Lys His Ile Cys Ala
130 135 140

Ile Cys Gly Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr
145 150

Ser Cys Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Ile Arg
155 160 165

Lys Asp Leu Ile Thr Thr Cys Arg Asp Asn Lys Asp Cys Leu
170 175 180

Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln Tyr Cys Arg Tyr
185 190 195

Gln Lys Cys Leu Val Met Gly Met Lys Arg Glu Ala Val Gln
200 205 210

Gln Gln Arg Gln Arg Ser Arg Glu Arg Ala Glu Ser Glu Ala
215 220

Glu Cys Ala Thr Ser Gly His Glu Asp Met Pro Val Glu Arg
225 230 235

Ile Leu Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Ser
240 245 250

Tyr Gly Asp Met Asn Met Glu Asn Ser Thr Asn Asp Pro Val
255 260 265

Thr Asn Ile Cys His Ala Ala Asp Lys Gln Leu Phe Thr Leu
270 275 280

Val Glu Trp Ala Lys Arg Ile Pro His Phe Ser Asp Leu Thr
285 290

Leu Glu Asp Gln Val Ile Leu Leu Arg Ala Gly Trp Asn Glu
295 300 305

Leu Leu Ile Ala Ser Phe Ser His Arg Ser Val Ser Val Gln
310 315 320

Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Ser
325 330 335

Ser Ala His Ser Ala Gly Val Gly Ser Ile Phe Asp Arg Val
340 345 350

Leu Thr Glu Leu Val Ser Lys Met Lys Asp Met Gln Met Asp
355 360

Lys Ser Glu Leu Gly Cys Leu Arg Ala Ile Val Leu Phe Asn
365 370 375

Pro Asp Ala Lys Gly Leu Ser Asn Pro Ser Glu Val Glu Thr
380 385 390

Leu Arg Glu Lys Val Tyr Ala Thr Leu Glu Ala Tyr Thr Lys
395 400 405

Gln Lys Tyr Pro Glu Gln Pro Gly Arg Phe Ala Lys Leu Leu
410 415 420

Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu
425 430

Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile
435 440 445

Asp Thr Phe Leu Met Glu Met Leu Glu Thr Pro Leu Gln Ile
450 455 460

Thr

Claims

1. Isolated, purified, or enriched nucleic acid comprising a contiguous nucleic acid sequence encoding hRXR-γ polypeptide.

2. A nucleic acid probe for the detection of nucleic acid encoding a hRXR-γ polypeptide in a sample.

3. Recombinant nucleic acid comprising a contiguous nucleic acid sequence encoding a hRXR-γ polypeptide, and a vector or a promoter effective to initiate transcription of said nucleic acid sequence in a host cell.

4. Recombinant nucleic acid comprising a transcriptional region functional in a cell, a sequence complimentary to an RNA sequence encoding a hRXR-γ polypeptide, and a transcriptional termination region functional in a cell.

5. An isolated, purified, recombinant, or enriched hRXR-γ polypeptide.

6. A purified antibody having specific binding affinity to a hRXR-γ polypeptide.

7. A hybridoma which produces an antibody having specific binding affinity to a hRXR-γ polypeptide.

8. A method of detecting a compound capable of binding to a hRXR-γ polypeptide comprising the steps of incubating said compound with said hRXR-γ polypeptide and detecting the presence of said compound bound to said hRXR-γ polypeptide.

9. The method of claim 8 wherein said compound is a retinoid.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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08/377,423 23 January 1995 (23.01.95) US

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(74) Agents: CHEN, Anthony, C. et al.; Lyon & Lyon, First Interstate World Center, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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(54) Title: HUMAN RETINOID X RECEPTOR - GAMMA (hRXR-GAMMA)

(57) Abstract

The present invention relates to a novel retinoid receptor, human retinoid X receptor γ. hRXRγ modulates transcription of certain genes in the presence of certain retinoid compounds. hRXRγ differs from known retinoid receptors in nucleotide sequence, amino acid sequence, and expression pattern in tissues. The invention provides isolated, purified, or enriched nucleic acid encoding hRXRγ polypeptides and vectors containing thereof, cells transformed with such vectors, and methods of screening for compounds capable of binding hRXRγ polypeptides. The invention also provides isolated, purified, enriched, or recombinant hRXRγ polypeptides, antibodies having specific binding affinity to hRXRγ polypeptides, and hybridomas producing such antibodies.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/00847

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C07K16/28 C12N5/12 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 15216 (INST NAT SANTE RECH MED ;CENTRE NAT RECH SCIENT (FR); UNIV PASTEUR) 5 August 1993 see page 68 - page 69 see page 16, line 28 - page 17, line 2 ---	1-4
X	GENOMICS 20 (3). 1994. 397-403. , XP000574901 ALMASAN A ET AL: "Chromosomal localization of the human retinoid X receptors." see page 398, left-hand column, line 13 - line 23 --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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1

Date of the actual completion of the international search
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Name and mailing address of the ISA
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Authorized officer
Maddox, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/00847

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUM. REPROD. (1994), 9(2), 229-34 , XP000574898 KUMARENDRAN, M. KUMAR ET AL: "Estrogen and progesterone do not regulate the expression of retinoic acid receptors and retinoid "X" receptors in human endometrial stromal cells in vitro" see page 230 left col. section ' Probes '	1,2
X	WO,A,91 12258 (SALK INST FOR BIOLOGICAL STUDI) 22 August 1991 see page 22, line 10 - line 30; claims 20-25	2,4
X	MOLECULAR ENDOCRINOLOGY 8 (7). 1994. 870-877. , XP000574900 TITCOMB M W ET AL: "Sensitive and specific detection of retinoid receptor subtype proteins in cultured cell and tumor extracts." see whole document particularly page 871 left col. para. 1	1,5-7
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, 15 December 1993, pages 26625-26633, XP002007755 ALLEGRETTO, E.A., ET AL.: "Transactivation properties of retinoic acid and retinoic X receptors in mammalian cells and yeast" see page 26626 left col. section ' Antibodies '	6 3,4
X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 204 (2). 1994. 525-536., XP002007756 ROCHETTE-EGLY C ET AL: "Detection of retinoid X receptors using specific monoclonal and polyclonal antibodies." see the whole document	6,7
P,X	EMBL SEQUENCE DATABASE.RELEASE 45. 10-NOV-1995. ACESSION NO. U38480, XP002007757 COOKE, T.A., ET AL.: "Human retinoid X receptor-gamma mRNA, complete cds." see sequence	1,5
A	WO,A,93 11755 (SALK INST FOR BIOLOGICAL STUDI ;BAYLOR COLLEGE MEDICINE (US); LIGA) 24 June 1993 see page 37, line 25 - page 41, line 12	1-9

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1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ENDOCRINOLOGY 135 (6). 1994. 2595-2607. , XP000575863 DOWHAN D H ET AL: "Identification of deoxyribonucleic acid sequences that bind retinoid-X receptor-gamma with high affinity." see the whole document ---	1-9
A	GUT 36 (2). 1995. 255-258. , XP000574906 KANE K F ET AL: "1,25-dihydroxyvitamin D-3 and retinoid X receptor expression in human colorectal neoplasms." see the whole document -----	1

INTERNATIONAL SEARCH REPORT

...information on patent family members

Inter...nal Application No
PCT/US 96/00847

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9315216	05-08-93	CA-A-	2106382	25-07-93
		EP-A-	0577814	12-01-94
		JP-T-	6510206	17-11-94
WO-A-9112258	22-08-91	AU-B-	1155395	25-05-95
		AU-B-	654270	03-11-94
		AU-B-	7338391	03-09-91
		CA-A-	2075182	10-08-91
		EP-A-	0514488	25-11-92
		JP-T-	5504479	15-07-93
WO-A-9311755	24-06-93	AU-B-	3421893	19-07-93
		CA-A-	2123223	24-06-93
		DE-T-	617614	08-06-95
		EP-A-	0617614	05-10-94
		ES-T-	2066750	16-03-95
		JP-T-	8502238	12-03-96